

CLAIMS

WE CLAIM:

- 5 1. A composition comprising a water-soluble label comprising a 2nd generation poly(amidoamine) dendrimer encapsulating a noble metal nanocluster, wherein the label has characteristic Raman bands, expressed in wavenumbers (cm⁻¹) as a shift in energy ranging from 100-3500 cm⁻¹ from the excitation laser energy.
- 10 2. A composition comprising a water-soluble label comprising a 4th generation poly(amidoamine) dendrimer encapsulating a noble metal nanocluster, wherein the label has characteristic Raman bands, expressed in wavenumbers (cm⁻¹) as a shift in energy ranging from 100-3500 cm⁻¹ from the excitation laser energy.
- 15 3. A composition comprising a water-soluble label comprising a peptide comprising a polypeptide sequence as defined in SEQ ID NO:1 encapsulating a noble metal nanocluster, wherein the label has characteristic Raman bands, expressed in wavenumbers (cm⁻¹) as a shift in energy ranging from 100-3500 cm⁻¹ from the excitation laser energy.
- 20 4. A composition comprising a water-soluble label comprising an encapsulated noble metal nanocluster, wherein the label has a single-molecule Raman spectrum.
- 25 5. The composition of Claim 4, wherein the noble metal nanocluster comprises between 2 and 8 noble metal atoms.
- 30 6. The composition of Claim 4, wherein the noble metal is gold.
- 35 7. The composition of Claim 4, wherein the noble metal is silver.
8. The composition of Claim 4, wherein the noble metal is copper.
9. The composition of Claim 4, wherein the encapsulated noble metal nanocluster has a lifetime component of less than approximately 100 fs.
10. The composition of Claim 4, wherein the label has a single molecule anti-Stokes spectrum.

11. The composition of Claim 10, wherein the low excitation intensity is approximately 30 W/cm² at approximately 514 nm.

5 12. The composition of Claim 4, wherein the encapsulated noble metal nanocluster has a spectral emission that provides information about a biological state selected from the group consisting of a quantitative and qualitative presence of a biological moiety; structure, composition, and conformation of a biological moiety; localization of a biological moiety in an environment; an interaction between biological moieties; an alteration in structure of a biological compound; and an alteration in a cellular process.

10 13. The composition of Claim 4, wherein the noble metal nanocluster has a varying charge.

15 14. The composition of Claim 4, wherein the size of the encapsulated noble metal nanocluster is from approximately less than 1 nm to 15 nm in diameter.

20 15. The composition of Claim 4, wherein the absorption cross section of the encapsulated noble metal nanocluster is approximately $\sigma = 10^{-14}$ cm².

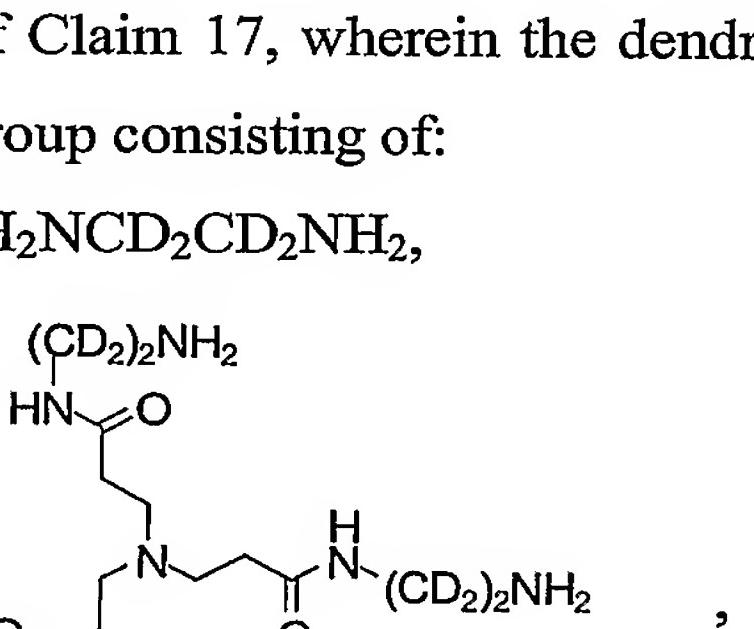
16. The composition of Claim 4, wherein the Raman cross section of the encapsulated noble metal nanocluster is approximately $\sigma = 10^{-14}$ cm².

25 17. The composition of Claim 4, wherein the noble metal nanocluster is encapsulated in a dendrimer.

18. The composition of Claim 17, wherein the dendrimer comprises a dendrimer core selected from the group consisting of:

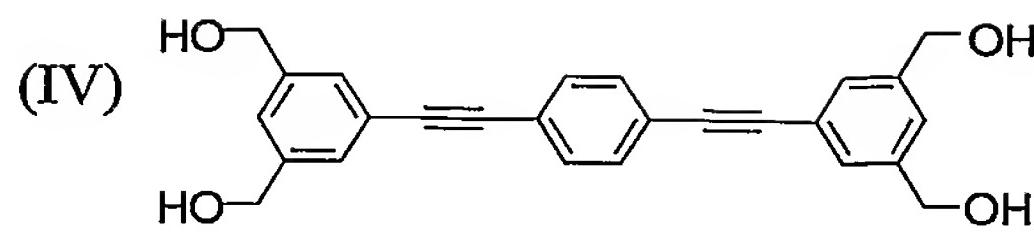
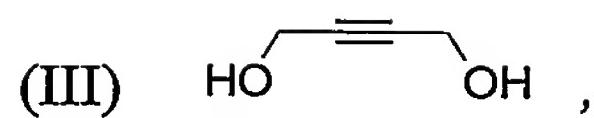
30 (I) H₂NCD₂CD₂NH₂,

(II)



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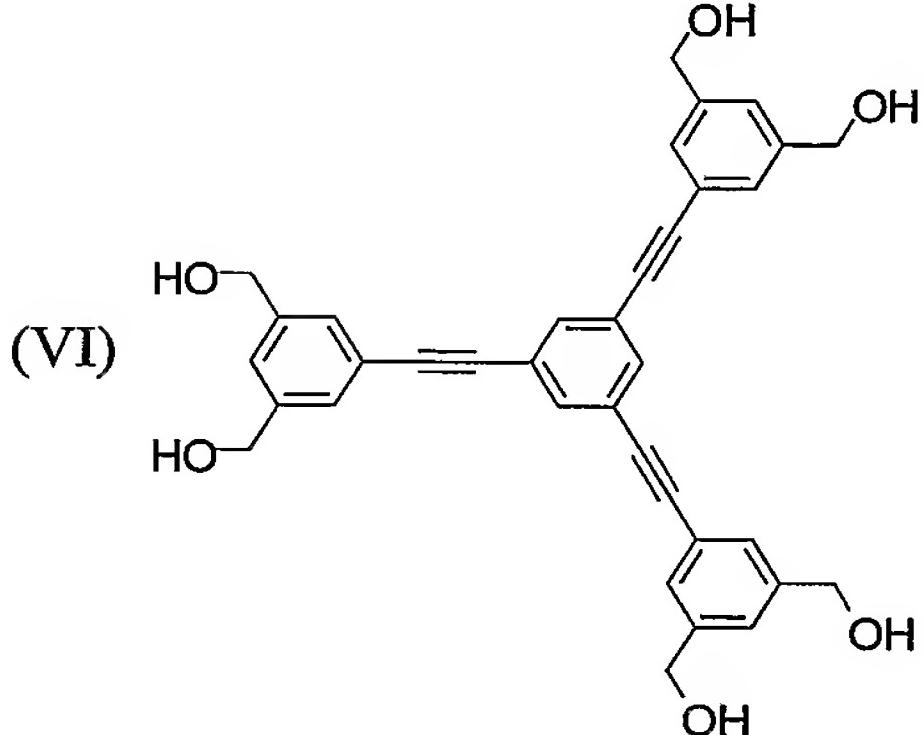
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19. The composition of Claim 17, wherein the dendrimer comprises poly(amidoamine).
20. The composition of Claim 19, wherein the poly(amidoamine) dendrimer is selected from the group consisting of a 0th generation, 1st generation, 2nd generation, 3rd generation, a 4th generation, and a higher generation poly(amidoamine) dendrimer.
25. 21. The composition of Claim 19, wherein the poly(amidoamine) dendrimer is a 2nd generation, or a 4th generation OH-terminated poly(amidoamine) dendrimer.
30. 22. The composition of Claim 4, wherein the noble metal nanocluster is encapsulated in a peptide.
35. 23. The composition of Claim 22, wherein the peptide is approximately 5-20 amino acids in length.
24. The composition of Claim 22, wherein the peptide comprises repeating amino acid dimers.

25. The composition of Claim 24, wherein the repeating amino acid dimers are alanine and histidine.
- 5 26. The composition of Claim 22, wherein the peptide comprises a polypeptide sequence as defined in SEQ ID NO:1.
27. The composition of Claim 4, wherein the encapsulated noble metal nanocluster further comprises a functional group having a single-molecule Raman spectrum.
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28. The composition of Claim 27, wherein the functional group is selected from the group consisting of C-D, C≡N, C≡C, and C≡O.
29. The composition of Claim 27, wherein the functional group has a vibrational frequency in the 1900~2300 cm⁻¹ spectral region.
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30. The composition of Claim 27, where the functional group is located in any generation of a dendrimer.
- 20 31. A composition comprising a water-soluble label comprising an encapsulated noble metal nanocluster, wherein the encapsulated noble metal nanocluster has a non-linear optical property.
32. The composition of Claim 31, wherein the non-linear optical property is second
25 harmonic generation.
33. The composition of Claim 31, wherein the noble metal nanocluster comprises between 2 and 8 noble metal atoms.
- 30 34. The composition of Claim 31, wherein the noble metal is silver.
35. The composition of Claim 31, wherein encapsulated noble metal nanocluster comprises a dendrimer encapsulated silver nanocluster.
- 35 36. The composition of Claim 35, wherein the dendrimer comprises a poly(amidoamine).

37. The composition of Claim 36, wherein the dendrimer is a 2nd generation poly(amidoamine) dendrimer.
38. The composition of Claim 35, wherein the nanocluster is excited at approximately 5 860 nm, and wherein an emission peak is observed at approximately 430 nm.
39. The composition of Claim 31, wherein the encapsulated noble metal nanocluster has a lifetime component of less than approximately 100 ps.
- 10 40. The composition of Claim 31, wherein the encapsulated noble metal nanocluster has a two-photon-excited emission at 860 nm having a shorter excited state lifetime in comparison to that resulting from single photon excitation at 430-nm.
- 15 41. The composition of Claim 31, wherein the encapsulated noble metal nanocluster has a two-photon-excited emission at 860 nm having the same excited state lifetime in comparison to that resulting from single photon excitation at 430-nm.
- 20 42. The composition of Claim 31, wherein the encapsulated noble metal nanocluster has a spectral emission that provides information about a biological state selected from the group consisting of a quantitative and qualitative presence of a biological moiety; structure, composition, and conformation of a biological moiety; localization of a biological moiety in an environment; an interaction between biological moieties; an alteration in structure of a biological compound; and an alteration in a cellular process.
- 25 43. The composition of Claim 31, wherein the size of the encapsulated noble metal nanocluster is from approximately less than 1 nm to 15 nm in diameter.
- 30 44. The composition of Claim 31, wherein a two-photon fluorescence cross section of the encapsulated noble metal nanocluster is greater than approximately 10⁵ GM.
45. A composition comprising a water-soluble fluorescent label comprising an oligonucleotide encapsulated noble metal nanocluster.
- 35 46. The composition of Claim 45, wherein the noble metal nanocluster comprises between 2 and 8 noble metal atoms.

47. The composition of Claim 45, wherein the noble metal is gold.
48. The composition of Claim 45, wherein the noble metal is silver.
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49. The composition of Claim 45, wherein the noble metal is copper.
50. The composition of Claim 45, wherein the encapsulated noble metal nanocluster has a fluorescence quantum yield of greater than approximately 1% and has a
10 saturation intensity ranging from approximately 1 to 10^6 W/cm².
51. The composition of Claim 50, wherein the low excitation intensity is approximately 30 W/cm² at approximately 460 nm.
- 15 52. The composition of Claim 45, wherein the encapsulated noble metal nanocluster exhibits a polarized spectral emission and exhibits a dipole emission pattern.
53. The composition of Claim 45, wherein the encapsulated noble metal nanocluster has a spectral emission that provides information about a biological state selected
20 from the group consisting of a quantitative and qualitative presence of a biological moiety; structure, composition, and conformation of a biological moiety; localization of a biological moiety in an environment; an interaction between biological moieties; an alteration in structure of a biological compound; and an alteration in a cellular process.
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54. The composition of Claim 45, wherein the noble metal nanocluster has a varying charge.
55. The composition of Claim 45, wherein the size of the encapsulated noble metal
30 nanocluster is from approximately less than 1 nm to 15 nm in diameter.
56. The composition of Claim 45, wherein the noble metal nanocluster emits greater than approximately 10^8 photons before photobleaching.
- 35 57. The composition of Claim 45, wherein when the composition comprising more than one noble metal nanocluster is excited, greater than approximately 70% of the noble metal nanoclusters fluoresce for greater than approximately 10 minutes.

58. The composition of Claim 45, wherein the oligonucleotide is from approximately 1-200 nucleotides in length.
- 5 59. The composition of Claim 45, wherein the oligonucleotide is from approximately 10-35 nucleotides in length.
60. The composition of Claim 45, wherein the oligonucleotide comprises a polyA, polyG, polyT or polyC sequence.
- 10 61. The composition of Claim 45, wherein the oligonucleotide comprises a nucleotide sequence as defined in SEQ ID NO:2.
62. The composition of Claim 45, wherein the oligonucleotide comprises a nucleotide sequence as defined in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID 15 NO:6, SEQ ID NO:7 or SEQ ID NO:8.
63. The composition of Claim 45, wherein one noble metal nanocluster binds to the oligonucleotide, and wherein the encapsulated nanocluster comprises 4 or fewer noble metal atoms.
- 20 64. A method of preparing an oligonucleotide encapsulated noble metal nanocluster capable of fluorescing, comprising the steps of:
 - a) combining an oligonucleotide, an aqueous solution comprising a noble metal, and distilled water to create a combined solution;
 - 25 b) adding a reducing agent;
 - c) subsequently adding a sufficient amount of an acidic compound to adjust the combined solution to a neutral range pH; and
 - d) mixing the pH adjusted, combined solution to allow the formation of the oligonucleotide encapsulated noble metal nanocluster.
- 30 65. The method of Claim 64, wherein the reducing agent is selected from the group consisting of light, a chemical reducing agent, a photochemical reducing agent and a combination thereof.
- 35 66. The method of Claim 64, wherein the noble metal to oligonucleotide molar ratio in step a) is approximately 0.1:1.

67. The method of Claim 64, wherein the temperature of the combined solution is between approximately 18°C to approximately 38°C from step a) through step c).
- 5 68. The method of Claim 64, wherein the temperature of the combined solution is between approximately 20°C to approximately 23°C.
69. The method of Claim 64, wherein the noble metal is selected from the group consisting of silver, gold, and copper.
- 10 70. The method of Claim 64, wherein the aqueous solution comprising a noble metal is selected from the group consisting of AgNO₃, HAuCl₄•nH₂O, and CuSO₄•nH₂O.
71. The method of Claim 64, wherein the oligonucleotide is from approximately 10-35 nucleotides in length.
- 15 72. The method of Claim 64, wherein the oligonucleotide comprises a polyA, polyG, polyT or polyC sequence.
- 20 73. The method of Claim 64, wherein the oligonucleotide comprises a nucleotide sequence as defined in SEQ ID NO:2.
74. The method of Claim 64, wherein the oligonucleotide comprises a nucleotide sequence as defined in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 or SEQ ID NO:8.
- 25 75. The method of Claim 64, wherein the size of the oligonucleotide encapsulated noble metal nanocluster is from approximately less than 1 nm to approximately 15 nm in diameter.
- 30 76. The method of Claim 64, wherein the oligonucleotide encapsulated noble metal nanocluster is capable of fluorescing over a pH range of approximately 3 to 9.
77. The method of Claim 64, wherein the oligonucleotide encapsulated noble metal nanocluster emits greater than approximately 10⁶ photons before photobleaching.

78. The method of Claim 64, wherein when more than one oligonucleotide encapsulated noble metal nanocluster is excited, greater than approximately 70% of the noble metal nanoclusters fluoresce for greater than approximately 10 minutes at a continuous excitation energy of approximately 300 W/cm² at 514.5 nm or 476 nm.

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